
Sequence Listing was accepted.

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Reviewer: Durreshwar Anjum

Timestamp: [year=2010; month=1; day=20; hr=11; min=41; sec=50; ms=556;]

Validated By CRFValidator v 1.0.3

Application No: 10580635 Version No: 2.0

Input Set:

Output Set:

Started: 2010-01-04 17:43:00.033

Finished: 2010-01-04 17:43:03.054

Elapsed: 0 hr(s) 0 min(s) 3 sec(s) 21 ms

Total Warnings: 30

Total Errors: 0

No. of SeqIDs Defined: 30

Actual SeqID Count: 30

Error code		Error Description
W	402	Undefined organism found in <213> in SEQ ID (1)
W	402	Undefined organism found in <213> in SEQ ID (2)
W	402	Undefined organism found in <213> in SEQ ID (3)
W	402	Undefined organism found in <213> in SEQ ID (4)
W	213	Artificial or Unknown found in <213> in SEQ ID (5)
W	213	Artificial or Unknown found in <213> in SEQ ID (6)
W	213	Artificial or Unknown found in <213> in SEQ ID (7)
W	213	Artificial or Unknown found in <213> in SEQ ID (8)
W	213	Artificial or Unknown found in <213> in SEQ ID (9)
W	213	Artificial or Unknown found in <213> in SEQ ID (10)
W	213	Artificial or Unknown found in <213> in SEQ ID (11)
W	213	Artificial or Unknown found in <213> in SEQ ID (12)
W	213	Artificial or Unknown found in <213> in SEQ ID (13)
W	213	Artificial or Unknown found in <213> in SEQ ID (14)
W	213	Artificial or Unknown found in <213> in SEQ ID (15)
W	213	Artificial or Unknown found in <213> in SEQ ID (16)
W	213	Artificial or Unknown found in <213> in SEQ ID (17)
W	213	Artificial or Unknown found in <213> in SEQ ID (18)
W	213	Artificial or Unknown found in <213> in SEQ ID (19)
W	213	Artificial or Unknown found in <213> in SEQ ID (20)

Input Set:

Output Set:

Started: 2010-01-04 17:43:00.033 **Finished:** 2010-01-04 17:43:03.054

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Total Warnings: 30
Total Errors: 0

No. of SeqIDs Defined: 30

Actual SeqID Count: 30

Error code		Error Description				
W	213	Artificial or Unknown found in <213> in SEQ ID (21)				
W	213	Artificial or Unknown found in <213> in SEQ ID (22)				
W	213	Artificial or Unknown found in <213> in SEQ ID (23)				
W	402	Undefined organism found in <213> in SEQ ID (24)				
W	213	Artificial or Unknown found in <213> in SEQ ID (25) This error has occured more than 20 times, will not be displayed				

SEQUENCE LISTING

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<110> Pastan, Ira H.
      Ho, Mitchell
      Bang, Sook-Hee
      The Government of the United States
         as represented by The Secretary of the
         Department of Health and Human Services
<120> Mutated Anti-CD22 Antibodies and Immunoconjugates
<130> 015280-500100US
<140> 10580635
<141> 2010-01-04
<150> US 60/525,371
<151> 2003-11-25
<150> WO PCT/US04/39617
<151> 2004-11-24
<160> 30
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<223> RFB4 mouse IgG1 anti-human CD22 monoclonal
      antibody light chain variable region (VL)
<220>
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      antibody light chain variable region (VL)
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                                                                   48
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1
                                                          15
gac aga gtc acc att agt tgc agg gca agt cag gac att agc aat tat
                                                                   96
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
             20
tta aac tgg tat cag cag aaa cca gat gga act gtt aaa ctc ctg atc
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
         35
                                                  45
                             40
tac tac aca tca ata tta cac tca gga gtc cca tca agg ttc agt ggc
                                                                   192
Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
```

50 55 60

agt ggg tct gga aca gat tat tct ctc acc att agc aac ctg gag caa 240 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln 65 70 75 80

gaa gat ttt gcc act tac ttt tgc caa cag ggt aat acg ctt ccg tgg 288
Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
85 90 95

acg ttc ggt gga ggc acc aag ctg gaa atc aaa 321
Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 2

<211> 107

<212> PRT

<213> Mus sp.

<220>

<223> RFB4 mouse IgG1 anti-human CD22 monoclonal antibody light chain variable region (VL)

<400> 2

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly

1 5 10 15

Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$

Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly $50 \ 55 \ 60$

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln 65 70 75 80

Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp

85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys 100 105

<210> 3

<211> 369

<212> DNA

<213> Mus sp.

<220>

<223> RFB4 mouse IgG1 anti-human CD22 monoclonal antibody heavy chain variable region (VH)

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<223> RFB4 mouse IgG1 anti-human CD22 monoclonal
      antibody heavy chain variable region (VH)
<400> 3
gaa gtg cag ctg gtg gag tct ggg gga ggc tta gtg aag cct gga ggg
                                                                   48
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
tee etg aaa ete tee tgt gea gee tet gga tte get tte agt ate tat
                                                                   96
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
             20
                                 25
gac atg tct tgg gtt cgc cag act ccg gag aag agg ctg gag tgg gtc
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val
         35
                             40
gca tac att agt agt ggt ggt acc acc tac tat cca gac act gtg
                                                                   192
Ala Tyr Ile Ser Ser Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
     50
                         55
aag ggc cga ttc acc atc tcc aga gac aat gcc aag aac acc ctg tac
                                                                   240
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
                     70
ctg caa atg agc agt ctg aag tct gag gac aca gcc atg tat tac tgt
                                                                   288
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
                 85
                                     90
gca aga cat agt ggc tac ggt agt agc tac ggg gtt ttg ttt gct tac
Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
            100
                                105
tgg ggc caa ggg act ctg gtc act gtc tct gca
                                                                   369
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
        115
                            120
<210> 4
<211> 123
<212> PRT
<213> Mus sp.
<220>
<223> RFB4 mouse IgG1 anti-human CD22 monoclonal
      antibody heavy chain variable region (VH)
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile Tyr
             20
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Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Val

45

40

35

<221> CDS

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Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Val
     50
                         55
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
                     70
                                          75
65
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
            100
                                105
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala
                            120
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<211> 4
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<213> Artificial Sequence
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<223> Description of Artificial Sequence:carboxyl
      terminal fragment binding KDEL recycling receptor
      for transport of construct into cytosol from
      endoplasmic reticulum
<400> 5
Lys Asp Glu Leu
 1
<210> 6
<211> 4
<212> PRT
<213> Artificial Sequence
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<223> Description of Artificial Sequence:carboxyl
      terminal fragment binding KDEL recycling receptor
      for transport of construct into cytosol from
      endoplasmic reticulum
<400> 6
Arg Glu Asp Leu
 1
<210> 7
<211> 6
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<213> Artificial Sequence
<220>
<223> Description of Artificial Sequence: RFB4 variable
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light chain (VL) complementarity determining

```
region 1 (CDR1)
<400> 7
Gln Asp Ile His Gly Tyr
<210> 8
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<213> Artificial Sequence
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      light chain (VL) complementarity determining
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<400> 8
Gln Asp Ile Gly Arg Tyr
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<211> 6
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<213> Artificial Sequence
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      light chain (VL) complementarity determining
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Gln Asp Ile Arg Gly Tyr
<210> 10
<211> 6
<212> PRT
<213> Artificial Sequence
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      light chain (VL) complementarity determining
      region 1 (CDR1)
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Gln Asp Ile Ala Arg Tyr
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<210> 11
<211> 3
<212> PRT
<213> Artificial Sequence
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<220>
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      light chain (VL) complementarity determining
      region 2 (CDR2)
<400> 11
Tyr Thr Ser
 1
<210> 12
<211> 9
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      region 3 (CDR3)
<400> 12
Gln Gln Gly Asn Thr Leu Pro Trp Thr
 1
                  5
<210> 13
<211> 8
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<213> Artificial Sequence
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      region 1 (CDR1)
<400> 13
Gly Phe Ala Phe Ser Ile Tyr Asp
 1
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      heavy chain (VH) complementarity determining
      region 2 (CDR2)
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Ile Ser Ser Gly Gly Gly Thr Thr
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<211> 16

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      heavy chain (VH) complementarity determining
      region 3 (CDR3)
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Ala Arg His Ser Gly Tyr Gly Ser Ser Tyr Gly Val Leu Phe Ala Tyr
                 5
                                     1.0
                                                          15
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                                     10
                                                          15
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      heavy chain (VH) complementarity determining
      region 3 (CDR3)
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                5
                                   10
                                                         15
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<212> PRT
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<223> Description of Artificial Sequence: RFB4 variable
      heavy chain (VH) complementarity determining
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Ala Arg His Ser Gly Tyr Gly Thr Thr Trp Gly Val Leu Phe Ala Tyr
                  5
                                     10
                                                          15
```

<212> PRT

```
<210> 19
<211> 16
<212> PRT
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     heavy chain (VH) complementarity determining
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Ala Arg His Ser Gly Tyr Gly Ser Thr Tyr Gly Val Leu Phe Ala Tyr
                                   10
<210> 20
<211> 107
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<213> Artificial Sequence
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<223> Description of Artificial Sequence:mutated RFB4 VL
     chain
<400> 20
Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
                           10
Asp Arg Val Thr Ile Ser Cys Arg Ala Ser Gln Asp Ile His Gly Tyr
            20
                              25
Leu Asn Trp Tyr Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
        35
                           40
                                               45
Tyr Tyr Thr Ser Ile Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
     50
                        55
                                            60
Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Gln
                                        75
Glu Asp Phe Ala Thr Tyr Phe Cys Gln Gln Gly Asn Thr Leu Pro Trp
               85
                                    90
Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys
          100 105
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<212> PRT
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<223> Description of Artificial Sequence:mutated RFB4 VH
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chain

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Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Lys Pro Gly G	LУ							
1 5 10 15								
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ala Phe Ser Ile T	/r							
20 25 30								
	_							
Asp Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu Glu Trp Va	ı⊥							
35 40 45								
Alo Tun Ilo Con Con Clu Clu Clu The The Tun Tun Dec Ace The W	. 1							
Ala Tyr Ile Ser Ser Gly Gly Gly Thr Thr Tyr Tyr Pro Asp Thr Va 50 55 60	11							
33								
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu T	1r							
	30							
Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cy	/S							
85 90 95								
Ala Arg His Ser Gly Tyr Gly Thr His Trp Gly Val Leu Phe Ala Ty	/r							
100 105 110								
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala								
115 120								
<210> 22								
<211> 345								
<212> PRT								
<213> Artificial Sequence								
.000								

<220>

<223> Description of Artificial Sequence:Pseudomonas exotoxin A cytotoxic fragment PE38 translocating and ADP ribosylating domains

<400> 22

Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro 1 5 10 15

Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu
20 25 30

Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala $35 \hspace{1.5cm} 40 \hspace{1.5cm} 45$

Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu 50 55 60

Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln 65 70 75 80

Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Ala Glu Ser Glu 85 90 95

Arg Phe Val Arg Gln Gly Thr Gly Asn Asp Glu Ala Gly Ala Ala Asn 100 105 110

Gly Pro Ala Asp Ser Gly Asp Ala Leu Leu Glu Arg Asn Tyr Pro Thr 115 120 125

Gly Ala Glu Phe Leu Gly Asp Gly Gly Asp Val Ser Phe Ser Thr Arg 130 135 140

Gly Thr Gln Asn Trp Thr Val Glu Arg Leu Leu Gln Ala His Arg Gln 145 150 155 160

Leu Glu Glu Arg Gly Tyr Val Phe Val Gly Tyr His Gly Thr Phe Leu 165 170 175

Glu Ala Ala Gln Ser Ile Val Phe Gly Gly Val Arg Ala Arg Ser Gln 180 185 190

Asp Leu Asp Ala Ile Trp Arg Gly Phe Tyr Ile Ala Gly Asp Pro Ala 195 200 205

Leu Ala Tyr Gly Tyr Ala Gln Asp Gln Glu Pro Asp Ala Arg Gly Arg
210 215 220

Ile Arg Asn Gly Ala Leu Leu Arg Val Tyr Val Pro Arg Ser Ser Leu 225 230 235 240

Pro Gly Phe Tyr Arg Thr Ser Leu Thr Leu Ala Ala Pro Glu Ala Ala 245 250 255

Gly Glu Val Glu Arg Leu Ile Gly His Pro Leu Pro Leu Arg Leu Asp
260 265 270

Ala Ile Thr Gly Pro Glu Glu Glu Gly Gly Arg Leu Glu Thr Ile Leu 275 280 285

Gly Trp Pro Leu Ala Glu Arg Thr Val Val Ile Pro Ser Ala Ile Pro 290 295 300

Thr Asp Pro Arg Asn Val Gly Gly Asp Leu Asp Pro Ser Ser Ile Pro 305 310 315 320

Asp Lys Glu Gln Ala Ile Ser Ala Leu Pro Asp Tyr Ala Ser Gln Pro
325 330 335

Gly Lys Pro Pro Arg Glu Asp Leu Lys 340 345

<210> 23

<211> 345

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<213> Artificial Sequence

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<223> Description of Artificial Sequence:Pseudomonas exotoxin A cytotoxic fragment PE38 translocating and ADP ribosylating domains with Arg at position 222 of PE38 (position 490 of Pseudomonas exotoxin A) mutated to Ala

1	Λ	0>	23
< 4	· U	\cup	

- Gly Gly Ser Leu Ala Ala Leu Thr Ala His Gln Ala Cys His Leu Pro 1 5 10 15
- Leu Glu Thr Phe Thr Arg His Arg Gln Pro Arg Gly Trp Glu Gln Leu 20 25 30
- Glu Gln Cys Gly Tyr Pro Val Gln Arg Leu Val Ala Leu Tyr Leu Ala 35 40 45
- Ala Arg Leu Ser Trp Asn Gln Val Asp Gln Val Ile Arg Asn Ala Leu 50 55 60
- Ala Ser Pro Gly Ser Gly Gly Asp Leu Gly Glu Ala Ile Arg Glu Gln 65 70 75 80
- Pro Glu Gln Ala Arg Leu Ala Leu Thr Leu Ala Ala Glu Ser Glu
 85